CHROMOSOME NUMBERS AND RELATIONSHIPS IN *CHARA LEPTOPITYS* A. BR.[1]

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(Plate ii; three Text-figures.)

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Synopsis.

Chara leptopitys A. Br. First chromosome counts in this species from Western Australia and South Australia show 14 chromosomes, an expected number in the haplostephanae. The numbers 21 and 42 were also found in dividing antheridial filament cells in certain enlarged antheridia produced by otherwise normal plants with 14 chromosomes. The possible significance of this endopolyploid condition is discussed in relation to the initiation of polyploidy by means of a multiplication of genomes in gametes in the charophytes.

In a collection of *Chara leptopitys* A. Br. from Western Australia there were found within single individuals two or three distinct chromosome numbers revealing a striking instance of endopolyploidy in these plants. A multiplication of genomes in dividing or extra large nuclei has been observed in occasional antheridial filament cells of naturally occurring, untreated *Chara contraria* (Hotchkiss, 1958), and *Nitella furcata* (Imahori and Kato, 1961). Endopolyploidy in *Chara leptopitys*, however, involves the cells of entire antheridial filaments and entire antheridia dividing in synchronous mitoses.

Materials and Methods.

Material of *Chara leptopitys* used in this study was from the extensive collections of charophytes made by Prof. R. D. Wood as reported by Wood (1962*b*, 1963, and in press) and Hotchkiss (1963, and in press). Among these collections from the Australasian area was a series of specimens fixed in the field for cytological examination and sent to the present author for study. The data recorded by Wood for the two collections used here are as follows:

1. R. D. Wood 60–10–11–1–B. In 2–4″ of water, muck bottom. Pool, W side of road 12 miles N of Katanning, Western Australia. Abundant; cold water.
2. R. D. Wood and von der Borsch 60–9–22–3. Sept. 22, 1960. In 3–5″ of water; fresh water pool on W side of road. C. 10 mi. SE of Salt Creek, Coorong Region, South Australia.

For fixation, fertile young stem tips, or "heads", were selected in the field and placed in freshly prepared acetic-alcohol in the usual manner. These were later transferred to 70% alcohol and after shipping were stored under refrigeration until examined in aceto-orcein squash preparations.

Observations.

Macroscopic Features. The material of *Chara leptopitys* from Western Australia consisted of seven separate heads or stem tips (Heads 1, 2, etc.) each containing 3–5 whorls of branchlets. It is not known whether these heads were from the same or different plants. In the process of study much of the material was utilized and reduced to small fragments, but the following general description, which agrees well with the description provided by Nordstedt (1891), was made before dissection. *Stem cortication* diplostichous; *stipulodes* haplostephanous, apparently alternating with the branchlets (opposite according to Nordstedt, 1891), long, slender, with acute tips, stipulodes well developed at nodes bearing few basal gametangia but few or lacking at nodes with many

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basal gametangia which appear to replace them; *branchlets* 5–7 per whorl, with 3–4 branchlet nodes including the terminal one, the lower two branchlet nodes fertile; *gametangia* dioecious (only male plants seen), *antheridia* aggregate at the base of the branchlets where they extend outside and below the branchlets, geminate or up to four per node at the two branchlet nodes (above the base) or occasionally solitary at the uppermost fertile node, antheridia 950μ in diameter, often with the plates as outwardly bulging protuberances.

The heads were examined in detail and their structures noted separately as follows. *Head 1* appeared abnormal in that there were many antheridia of different sizes clustered together at the base of the older whorls of branchlets. Besides a considerable range in antheridial size from small to large, surface protuberances were conspicuous on the larger antheridia. *Head 2* contained more antheridia, but the range in antheridial size was not much greater than normally seen within and between whorls of branchlets on the same plant. There were no extremely large, bulbous-looking antheridia. *Heads 3–7* bore normal appearing antheridia and generally resembled head 2.

The material of *Chara leptopitys* from South Australia was still more limited and consisted of but three heads with 4–5 whorls of branchlets bearing antheridia all of normal appearance. The stipulodes were longer, but in other respects this material resembled the Western Australian plants; the gametangia were in the same arrangement.

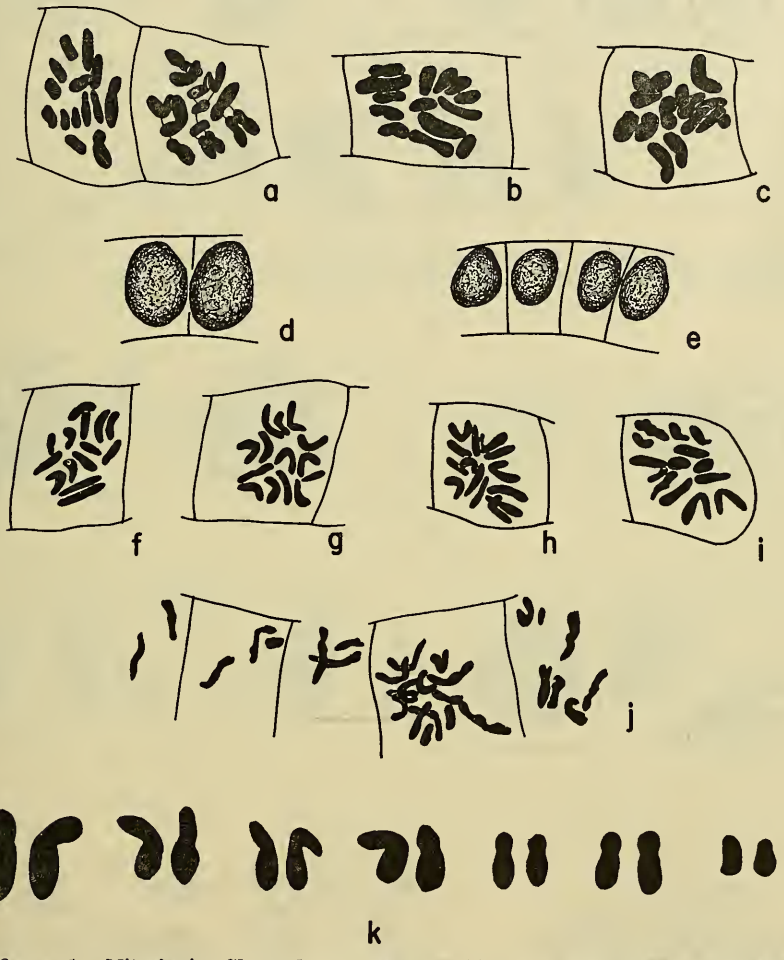
TABLE 1.

Dimensions of Antheridial Filament Cells of Chara leptopitys, *Western Australia.*

Chromosome Number.	Length and Breadth in Microns.			
	Resting Cell.	Dividing Cell.	Last Telophase Before Presperm.	Presperm Cell.
14	27–21	27–23	8–27	7–22
21	27–27	32–27	12–27	—
42	34–27	40–27	16–27	8–22

Chromosome Numbers and Cytological Observations. Because of the unusual appearance of the antheridia in one of the heads from Western Australia, this was examined first. All of the extraordinary chromosome numbers were observed in heads 1 and 2. The first preparation from head 1 was made with a selection of antheridia from the youngest apical whorl down through the third and oldest whorl of branchlets in order to sample the various stages of antheridial development. Antheridia in the first whorl appeared normal, were small, contained short, immature antheridial filaments with cells of a small diameter of 21μ (Table 1). Cells in mitotic division showed counts of 14 chromosomes only (Text-fig. 1; Plate ii, A). Antheridia from the second and third whorls ranged in size from small to large, the smaller antheridia had dividing filaments with 14 chromosome counts, and presperm cells and sperm of normal size. Some very large, bulbous, protuberant antheridia were entirely polyploid with all the dividing cells in all filaments with 42 chromosomes (Text-fig. 3; Plate ii, C). These cells were about 27μ broad by 38μ long when in division and somewhat shorter in interphase. The presperm and nearly mature sperm cells were all correspondingly large (Table 1). From the large number of adjacent synchronous stages of mitosis (up to 30–40 cells at metaphase and anaphase), the cells in 42-chromosome filaments appeared to be quite stable. At the same time, there were instances of irregularity in mitosis as seen in a tripolar spindle shown in Text-figure 3, *d*, which was bounded on either side by dividing cells in regular anaphase, and in other antheridial filament cells with either lobulate nuclei or with a multinucleate condition (Text-fig. 3, *e-f*).

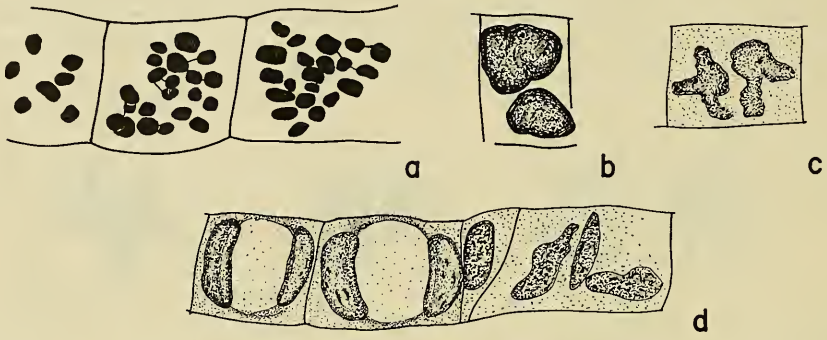
Alerted to the variability in the material, and with more antheridia available than in the first head, a second head was studied whorl by whorl in a series of five preparations. The first, from eight minute antheridia all from the youngest whorl, contained short, immature filaments in which all dividing cells displayed 14 chromosomes; no presperm or sperm cells were yet present. A second preparation from the second whorl of branchlets contained nine antheridia all normal in size and appearance. All but two of these showed normal presperm cells only; the remaining two antheridia had filament cells in division showing 14-chromosome counts only.



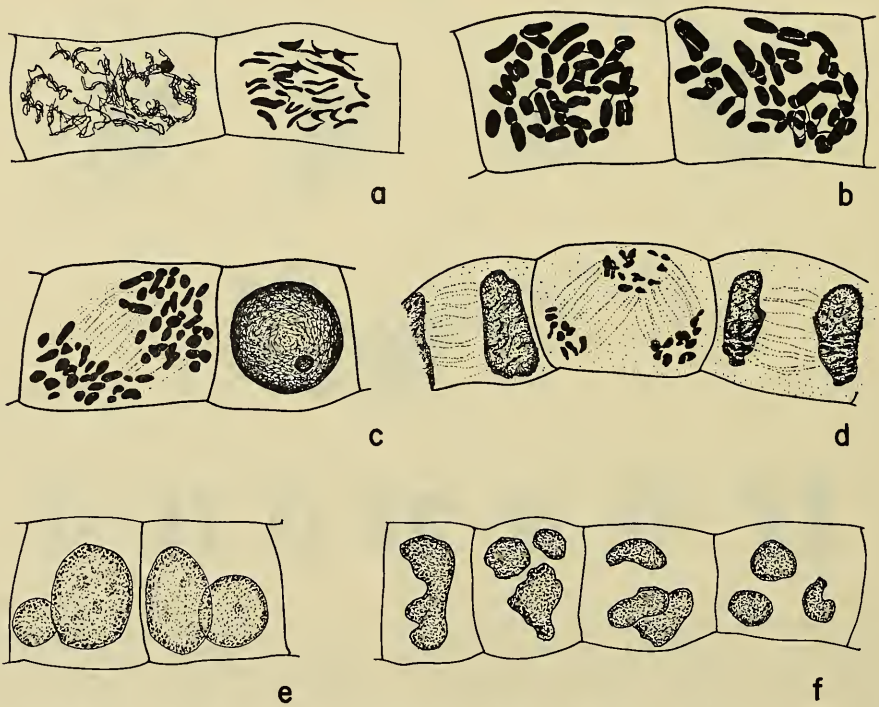
Text-figure 1.—Mitosis in *Chara leptopitys*, n = 14. *a-c*, Metaphase, showing somatic pairing in *a*. *d*, Last telophase preceding presperm stage. *e*, Telophase in presperm cells. *f-j*, Metaphase, showing knob-like projections in *j*. *k*, Paired arrangement of karyotype from Text-figure 1, *a*. *a-e*, Western Australia. *f-j*, South Australia. *a-j*, × 650. *k*, × 1300.

A third preparation from the third whorl of branchlets contained six antheridia, five of which were of the size normal for mature antheridia; the sixth one was slightly larger but not conspicuously bulbous. Four of the five antheridia showed normal presperm cells, the fifth one contained several filaments of normal size and with 14 chromosomes, but most filaments were made up of larger cells with 21 chromosomes in the dividing cells (Text-fig. 2; Plate ii, B). As many as 10 cells in synchronous, adjacent metaphase were seen, but usually only two adjacent cells were in metaphase. Presperm of both normal size and a larger size corresponding to the larger cells with

21 chromosomes were present, but no mature sperm were found in this antheridium. The sixth antheridium had all cells either as larger presperm or with 21-chromosome divisions. Antheridia in other preparations from whorl three were all of normal appearance and without divisions.



Text-figure 2.—Mitosis in *Chara leptopitys,* Western Australia, n = 21. *a,* Metaphase. *b-d,* Aberrant mitoses in terminal divisions, somatic reduction. All × 650.



Text-figure 3.—Mitosis in *Chara leptopitys,* Western Australia, n = 42. *a,* Prophase. *b,* Metaphase. *c,* Normal anaphase and telophase. *d,* Anaphase with tripolar spindle between normal anaphase figures. *e-f,* Telophase figures following aberrant mitoses, somatic reduction. All × 650.

In heads 3–7 from Western Australia and in the three heads of material from South Australia no unusual chromosome numbers were found. All heads were normal in both external and internal appearance and samplings of all revealed antheridial filaments with 14 chromosomes only.

The complement of 14 chromosomes (Text-fig. 1), found exclusively in the plants from South Australia and in the great majority of cases in the Western Australian plants, had no great range in size and under this treatment all chromosomes appeared to be relatively short and thick at metaphase. There was seen here, as reported in other species (Hotchkiss, in press), a notable degree of pairing between chromosomes of similar size and form (Text-fig. 1, *a*). Other pairs of similar chromosomes can be found in the metaphase grouping and the entire 14-chromosome complement can be arranged in pairs of at least approximately congruent chromosomes. The paired and unpaired chromosomes from Text-fig. 1, *a*, are thus arranged in seven pairs of similar chromosomes in Text-fig. 1, *k*. The occasional fine strands connecting chromosomes at metaphase were not confined to connections between chromosomes of the same size and form. The morphology of the chromosomes in *Chara leptopitys* from South Australia was somewhat different (Text-fig. 1, *f-j*) from those from Western Australia. In the former the overall diameter was smaller and there were present knob-like projections which may represent early anaphase separation of chromatids in the region of the centromere (Text-fig. 1, *j*). There was no evident ploidy, but in one of the heads some mature antheridia contained filaments with persistently dividing cells where only sperm or presperm cells would be expected.

Individual chromosomes in the 21- and 42-chromosome complements appeared to be equivalent to those in the 14-chromosome nuclei. These numbers suggest a multiplication of the genomes found in the 14-chromosome nucleus.

Discussion.

Chromosome Numbers. The haplontic life cycle in the Characeae consists of gametophyte plants comparable with the gametophyte phase of the bryophytes but, as in many of the algae, alternating with a one-celled sporophyte phase consisting of only the zygote. After a period of dormancy the first divisions of the zygote are meiotic and result in the growth of a new gametophyte phase, green and bearing gametangia at maturity. Following a series of mitotic divisions, the cells of the antheridial filaments are transformed directly into male gametes. Any chromosomal aberrations or variations developing and persisting in the maturation of the antheridial filaments are thus potentially carried by the gametes into the next generation. An alteration in chromosome number at this point in the life cycle may be the basis of one of the possible methods for the development of polyploidy.

Extensive polyploidy is now known to occur in the charophytes as a whole and has been reported by Telezynski (1929), Hotchkiss (1958, 1963, in press), Gillet (1959), Guerlesquin (1961), Imahori and Kato (1961), Tindall and Sawa (in press) and others. In addition, our unpublished data show an even greater importance of ploidy in the *Chara zeylanica* complex and in other species of *Chara* and *Nitella* than has been reported thus far. A degree of polyploidy induced by radiation and radiomimetic chemicals has been reported by Moutschen and Dahmen (1956) and Moutschen, Dahmen and Gillet (1956) in a series of experiments which we have repeated in part with the use of X-radiation.

In the genus *Chara* the series of polyploid gametic numbers 14, 28, 56 is found in some diplostephanous species groups and appears to be a sequence resulting from simple doubling of chromosome numbers. The number 14, probably derived from an ancestral 7, is the lowest number found in the extant species of *Chara*; no 7-chromosome species has been reported in this genus and probably none now exists. In the absence of detailed studies of meiosis, the best evidence for regarding the 7-chromosome genome as basic is found in studies revealing the common multiples of 7 in the polyploid species of *Chara*. Additional evidence may be found in several 14-chromosome forms in which there is a considerable degree of somatic pairing between chromosomes from two closely similar and presumably sometime homologous genomes of 7. A similar degree of somatic pairing can be seen in other species of *Chara* with higher chromosome number and in other genera as well. The denoting of 7 as ancestrally basic in *Chara* thus seems justified and to be useful in the interpretation

of speciation and evolutionary development in the genus. A theoretical basic 7 in *Chara* is comparable to the well-known low chromosome number of 6 in *Nitella*. At the same time it would appear that a diploidized 14 (2n = 28) may be the presently effective number in *Chara*. Polyploid numbers which are not multiples of 14 are extremely rare in species of *Chara* enumerated thus far.

Species with 21 chromosomes have not been reported in *Chara*, but the number 42 is found rather frequently in such groups as the *Chara zeylanica* complex. The theoretical number series 21 and 42 can be based on 7, and these numbers would represent triploid and hexaploid levels in the gametophyte respectively. One explanation of the origin of a 42-chromosome complement in *Chara* follows the pattern often suggested for triploids in higher plants, that is, resulting from a cross between two polyploid parents. In *Chara* this process would require parents at the 28 and 56 chromosome levels for a direct production of the hexaploid gametophyte with 42 chromosomes. Such parents do exist in some species groups and this possibility has been suggested (V. Proctor, personal communication) for certain forms in the *Chara zeylanica* complex. It may be possible also that the 42-chromosome level could be attained by a cross between parents with 14 and 28 chromosomes followed by a chromosome doubling or failure of reduction at germination of the zygote. From chromosome number relationships, such a possibility was suggested by Tindall and Sawa (in press) for *Chara aspera*, *Chara globularis* and *Chara delicatula* with 14, 28 and 42 chromosomes respectively. Since no 21-chromosome species is known to occur, the 21 is probably unbalanced genetically, whereas the 42-chromosome forms are stable and able to reproduce.

In *Chara leptopitys* the presence of 14 chromosomes in the great majority of antheridial filaments, and particularly in the younger antheridia of plants showing a partial polyploid condition in older stages, indicates that 14 is the present normal number for this species as it is for related species. The polyploid condition appears to be limited to certain antheridia; there is no reason to suppose that the apical portion of the plant as a whole, its meristem, or its separate vegetative parts are involved in the development of polyploid cells which appear in older regions only. Although the normal chromosome complement in *Chara leptopitys* is not at a high polyploid level, interest here lies in the suggestion of another possible site for the initiation of polyploidy. In the charophytes there thus seem to be two principal locations where multiplication of genomes may most readily occur. At meiosis a failure in reduction resulting in a simple doubling of chromosomes is probably much the more common method and directly produces the polyploid condition. More indirectly, polyploidy originating in a multiplication of genomes in mitoses leading to the maturation of gametes depends upon the union of a polyploid gamete with another ploided or unploided gamete. The resulting possibilities would include triploidy, tetraploidy and hexaploidy. In either case there is an opportunity for hybridization and the results may be alloploid or autoploid.

A potential for the initiation of polyploidy through the multiplication of genomes in antheridial filament development thus appears to be present in *Chara leptopitys*. This assumption is supported by the production of gametes in apparently polyploid filaments. It may be presumed that some of these gametes, especially those with 42 chromosomes, are viable and capable of fertilization and that here, as in the other species with occasional polyploid antheridial cells, mentioned earlier, there is seen a mechanism for the effective multiplication of genomes in gametes. A puzzling feature of the process in *Chara leptopitys*, however, is the apparent lack of a simple doubling of chromosomes and the consequent lack of any 28-chromosome complements in the antheridial filaments examined. Furthermore, the direct production within an originally diploid gametophyte of the triploid-hexaploid levels in a chromosome number series of 14–21–42 involves something other than a simple doubling. For this phenomenon an explanation based on observations of the entire mechanism involved would be desirable but unfortunately is not available at present.

Of the two higher numbers it may be assumed that the 42 results from a doubling of the 21. This is the simplest explanation and is in accord with the positioning of the 42-chromosome cells in the older antheridia. Thus in a series leading from 14 through 21 to 42 chromosomes, only the origin of the 21 would require a special explanation. Assuming a basic genome of seven chromosomes, one might speculate on a mechanism whereby in a 14-chromosome complement the two closely similar but not necessarily identical genomes are capable of a differential response resulting in a division of one but not the other. It is tempting to recall here the work of Hughes-Schrader (1948) on the males of two probably polyploid species of coccids in which single genomes act independently and aberrantly through meiosis I and II and finally disintegrate. Although in each case there is no resultant increase in chromosome number in the coccids, such as seems to be found in *Chara leptopitys*, this may be an interesting parallel in differential genome reaction.

Of the various numbers seen in antheridial filaments of *Chara leptopitys* the 14 and 42 appear to be most stable. The 21 was seen in but a few filaments each with only a few cells at metaphase in which the number could be determined. The scanty 21-chromosome filaments were scattered in antheridia otherwise made up of 14-chromosome filaments. The low incidence of 21s seems to indicate a low rate of initiation for this number. Once established, the 21 may rather quickly double into the more stable 42-chromosome level or disintegrate after a short series of progressively more aberrant nuclear divisions. The great stability of the 42-chromosome nuclei is attested by the presence in the larger antheridia of numerous filaments of regularly dividing cells at all stages of mitosis. Although rare, there was a degree of irregularity in cell division in the antheridia with 42 chromosomes. The lobulate nuclei, the tripolar spindle and multinucleate cells bespeak a type of somatic reduction which may be able to proceed through several successive divisions but which will not produce functional sperm. The lobulate nuclei seen here somewhat resemble internodal nuclei (Gillet, 1961), but their form appears to be related more to the polyploid nature of these cells than to the physiological requirements of internodal cells described by Gillet. Whatever the immediate results of the unusual cytological behaviour seen in this limited sampling of *Chara leptopitys*, it is concluded to be of considerable theoretical importance in the general survey of the cytotaxonomy of the charophytes.

Relationships. *Chara leptopitys* Braun, 1882, was reported by Nordstedt (1891) as being found in Victoria and Tasmania and also, as *C. leptopitys* subsp. *ebracteata* Nords., in Western Australia and Victoria. Wood and Imahori (1959) listed this species as occasional in Australia. This species was placed by Nordstedt (1891) between *Chara braunii* and *Chara scoparia*, and in the revision of Wood (1962*a*) much the same position was accorded it in Subgenus Charopsis, Section Agardhia, Subsection Agardhia along with *Chara fibrosa*, *C. pseudohydropitys*, *C. ecklonii* and *C. submollusca*.

It is clear that *Chara leptopitys* occupies an important position in the systematics of the group of species with basal gametangia. If these species can be considered a group, and this group is interpreted as a reductional series (based on considerations not elaborated here) leading to a gradual elimination of the cortex on branchlets and stems, *Chara leptopitys* remains as the member of the group primitively possessing cortication in any degree, and pointing to origins for the group among the corticated forms. Chromosome numbers greater than 14 are unusual among the haplostephanae and none has yet been reported among those species with basal gametangia. Despite the extraordinary polyploidy described in this study, the normal chromosome complement in *Chara leptopitys* is 14. Such a low number might be expected in this dioecious (heterothallic) species in accord with the observations (Hotchkiss, 1958) that in the Characeae dioecious species tend to have lower chromosome numbers while corresponding monoecious (homothallic) species have higher numbers. An assessment of the significance of the monoecious-dioecious states and their relationship to degree of polyploidy will be developed in future studies in the cytotaxonomy of the corticate and ecorticate haplostephanae, and will be based in part on the hypothesis of the derived nature of the dioecious state in the charophytes.

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EXPLANATION OF PLATE II.

Mitosis in *Chara leptopitys* A. Br. from Western Australia. A. Metaphase, n = 14. B, focal levels b-1 and b-2, Metaphase, n = 21. C, focal levels c-1, c-2, c-3, Metaphase, n = 42.